THE FIRST ANNUAL RISER LECTURE: MEIOFAUNA AND MICROBES—THE INTERACTIVE RELATIONS OF ANNELID HOSTS WITH THEIR SYMBIOTIC BACTERIA

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Abstract.—The gutless marine tubificid *Phallodrilus leukodermatus* (Oligochaeta) harbors two kinds of chemoautotrophic sulfur bacteria beneath its cuticle. The annelid hosts live predominantly around the redox potential discontinuity layer in calcareous sediments, providing their bacterial endosymbionts with access to both oxygen and reduced sulfur compounds. The bacteria produce energy by oxidation of these sulfur compounds, and use this energy for the fixation of CO_2 and production of carbohydrates. The worms utilize the exudates of the bacteria and also ingest a portion of the bacterial population in the epidermal cytoplasm. Further interactions in this apparently obligate symbiosis are discussed. The transmittance of the bacteria through the host generations by "infection" of freshly deposited eggs is correlated with a modified reproductive biology. The studies on this symbiosis reveal a well-coordinated balance between the annelid hosts and their extracellular bacterial partners.


Serendipity let us find a new species of small (2–3 cm long) white annelid worms in the calcareous sands of Bermudian reefs. These tubificid oligochaetes, described as *Phallodrilus leukodermatus* (Giere, 1979), were unique among known annelids. They lacked mouth, gut and anus and had no excretory organs. But the most striking feature was a thick, subcuticular 'mantle' of extracellular bacteria living between extensions of the epidermal cells (Fig. 1).

This bacterial association, present in each worm, even in the youngest, apparently consists of two different gram-negative prokaryotes (Fig. 2), a smaller, rod-shaped form (maximally 1.9×0.4 μm) with a thick cell envelope, and a larger oval one (about 3×2 μm) characterized by a thinner body wall and, more conspicuously, by the rich supply of vesicles in its cytoplasm (Giere et al. 1984).

This peculiar bacterial layer raises questions about the nutrition of these worms and about the structural implications of complete reduction of the gut in worms from sands that otherwise are richly populated by other meiofauna, including oligochaetes with normal guts. Also the microdistribution of these exceptional worms is quite unusual (Giere et al. 1982). We found rich populations in an area where, despite strong currents, anoxic horizons occurred at 5 cm sediment depth. Only a few worms were present in the upper, oxygenated strata, while the bulk of the population always occurred in the deeper layers where they were exposed to considerable amounts of toxic hydrogen
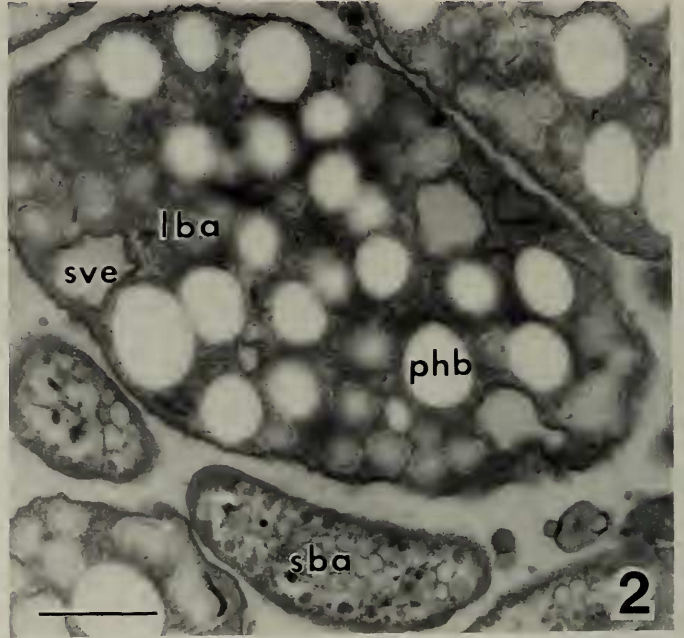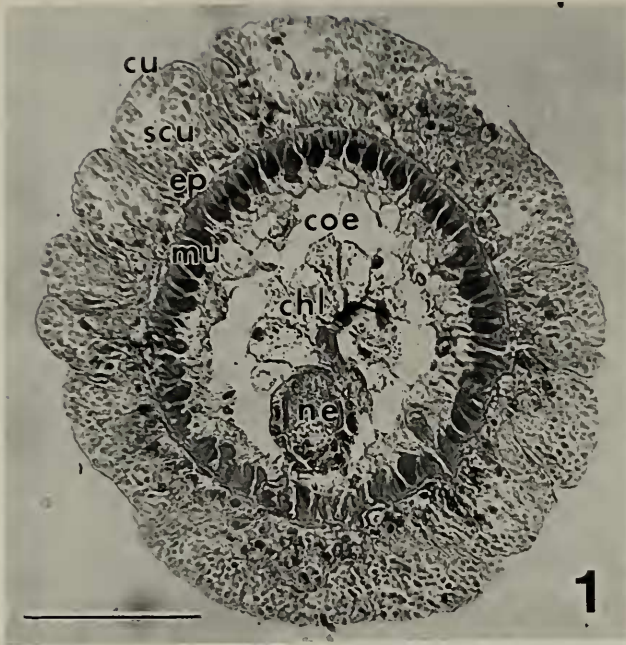
sulfide (around 100 μM S$^=$) with oxygen present only in minimal concentrations.

What promotes this exceptional distribution? How are the bacteria involved in this scenario? The answers require integration of morphological studies with ecological, physiological and microbiological analyses, i.e., consideration of the whole animal's biology. These studies were performed by a multidisciplinary team during several research periods at the Bermuda Biological Station for Research and in members' respective home laboratories.

Structural and ecological similarities suggest that the symbiotic prokaryotes are sulfur bacteria similar to those found in the giant vestimentiferan *Riftia* (Cavanaugh et al. 1981) from hydrothermal vents. The bacteria obtain energy by oxidation of reduced-sulfur compounds, enabling them to fix ambient CO_2 with subsequent production of carbohydrates.

For *Phallodrilus leukodermatus,* confirmation of this pathway results from physiological analyses: Ribulosebiphosphate-carboxylase in high activities in the bacteria enables a notable incorporation of CO_2 with a most rapid transfer into the malate pool (Felbeck et al. 1983). The high activity of enzymes like ATP-sulfurylase and sulfite oxidase, involved in the sulfur metabolism, indicates more specifically that sulfur compounds become metabolized by the symbiotic bacteria. Moreover, it can be shown that the products of this metabolism get stored in the numerous bacterial vesicles:

Energy-dispersive X-ray microanalysis demonstrates high sulfur peaks in membrane-bound bacterial vesicles, suggesting deposition of polysulfanes or polythionates as easily available reserve products (Steudel 1985, Steudel et al. 1987).

Sulfur also is found in the internal tissues of the worms where it accumulates in the "chloragosomes," annelid structures known to store xenobiotic substances. Thus, sulfur is transported from the ambient pore water (perhaps via the symbiotic bacteria?) to the internal worm tissues. Sulfate, the end product of oxidative sulfur metabolism, is excreted by the worms into the ambient sea water (Giere et al. 1988).

Bacteria oxidizing sulfur compounds are micro-aerophilic, gram-negative microorganisms thriving in those horizons where they have access to both reduced sulfur and oxygen, i.e., around the redox potential discontinuity layer. Microdistribution of the worm population presents ecological confirmation of these sulfur-based chemoautotrophic processes in the annelid symbionts. The bulk of the worm population is always concentrated around this transitional zone between the oxygenated surface layer and the anoxic depth with its poisonous hydrogen sulfide. In this zone the prokaryotic symbionts can synthesize their reserve substances. In addition to reduced-sulfur compounds, the bacterial cytoplasm abounds with unwalled globules (Fig. 2) filled with polyhydroxybutyric acid. Thus, in incident light worms from this zone have well-

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Figs. 1–3. *Phallodrilus leukodermatus*: 1, Cross section through the gutless worm with "mantle" of subcuticular bacteria, scale bar = 50 μm; chl = chloragocytes, coe = coelomic cavity, cu = cuticle, ep = epidermis, mu = musculature, ne = ventral nerve cord, scu = subcuticular space filled with bacteria; 2, The two kinds of bacteria endosymbionts; note difference in thickness of cell envelope, scale bar = 0.5 μm; lba = large bacterium, phb = vesicle filled with polyhydroxybutyric acid, sba = small bacterium, sve = sulfur vesicle; 3, Typical zonation pattern of subcuticular bacteria with free, active bacteria in peripheral position and enclosed, lytic bacteria in innermost position, scale bar = 5 μm; blys = bacteria in lysis, zba = zone of (free) bacteria, zint = intermediate zone bacteria become enclosed by epidermal strands, zlys = zone of bacterial lysis: bacteria are enclosed in vacuoles of epidermal cells and become digested (Fig. 1: light microscopy; Figs. 2+3: transmission electron microscopy).

fed bacteria and appear shiny white due to the rich content of light-refractile substances in the bacterial cytoplasm, which can be almost 6% of the worm's dry weight (Giere et al. 1988). When kept in pure, oxygenated seawater, the worms (through their bacteria) continue to take up CO_2 for a while from the environment, using up energy stored in the reserve substances. However, this uptake decreases soon, the worms become (after about 24 h) transparent pale-grayish, indicating rapid loss of their reserves, which in these "pale worms" is only 1.7% of their dry weight (Giere et al. 1988).

These results emphasize the biological interactions between the oligochaete host and its prokaryotic symbionts and lead to questions for the biological function of the bacteria/host system. The presence of bacteria in all members of the population and the ubiquity of bacterial incorporation in gutless oligochaete species—several species mainly from the Caribbean, but also from Australian reefs have been examined—suggests an obligate symbiosis with mutual benefits.

What are the benefits to the microorganisms of this incorporation into the worms' bodies?

—In a changing sedimentary system with steep, overlapping gradients of oxygen, sulfide, thiosulfate and CO_2, the highly motile worms are able to quickly adjust position relative to environmental fluctuations, maintaining an optimal combination of gradients required for growth of sulfur-oxidizing bacteria.

—It is conceivable that the prokaryotes take up organic substances excreted by their host which, in turn, has lost its excretory organs. The highly vesiculate epidermal cytoplasm of the worms and the multitude of membrane-bound secretory granules found extracellularly in their subcuticular lumen suggest this physiological pathway.

—The favorable position of the bacteria underneath the cuticle affords both shelter and easy access to nutrients from the ambient pore water through the highly permeable cuticle.

On the other hand, what benefits might the oligochaetes derive from close association with bacteria?

—Since the bacterial oxidation of reduced sulfur combines production of energy with detoxification of poisonous hydrogen sulfide, the microorganisms enhance the chances of the worms to invade a biotope of high ecological potential, rich in dissolved nutrients, but with little competition or predation. Our physiological experiments demonstrate that the worms can actively and selectively take up dissolved hexoses (particularly glucose and galactose) and acidic free amino acids (particularly glutamic acid and aspartate). These purely transcuticular absorption processes seem so intensive that, compared to corresponding figures from pogonophorans of the same size, they "contribute significantly" to the nutrition of *P. leukodermatus* (Liebezeit et al. 1983, Liebezeit, pers. comm.).

—Exuded bacterial products could serve as a valuable and ever-present source of further dissolved organic matter.

—Digestion of a certain portion of the internal bacterial population would provide an additional trophic pathway. Comparison of bacteria in the deeper layer of the epidermis with those in the subcuticular areas reveals a zonation pattern (Fig. 3): Peripheral bacteria are extracellular in a subcuticular lumen, where they accumulate reserve substances and multiply intensively by division. In the middle zone they tend to be surrounded by strands of the epidermal cells. the innermost bacteria are always found enclosed in intracellular vacuoles, apparently "engulfed" by the epidermal cells, and clearly undergoing lysis (Giere and Langheld 1987).

This coordinated system, combining bacterial growth and multiplication with lysis and digestion, is similar to those described
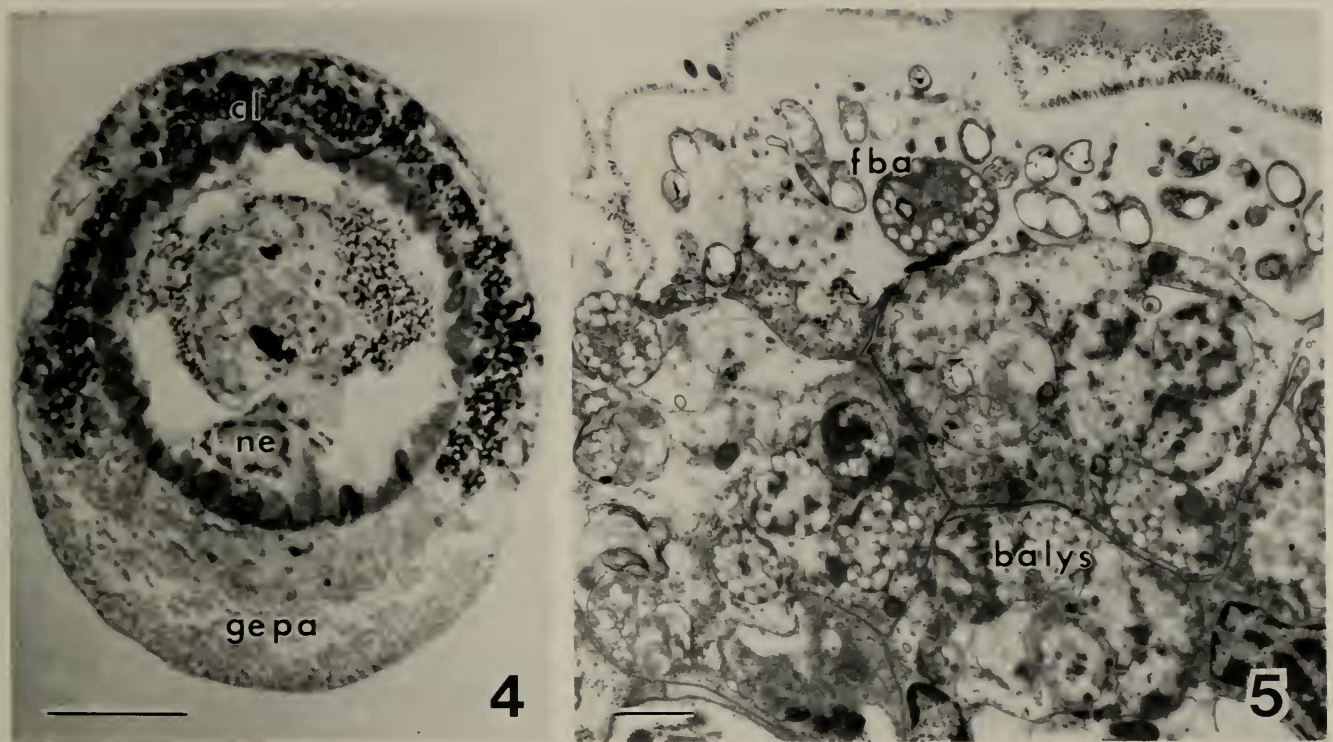
Figs. 4–5. *Phallodrilus leukodermatus*: 4, Cross section through genital pad in clitellar region, scale bar = 50 μm; cl = clitellar glands in epidermis, gepa = genital pad filled with bacteria, ne = ventral nerve cord; 5, Cross section through young embryo with some free subcuticular bacteria (both kinds) and many lytic bacteria enclosed in host cells, scale bar = 1 μm; balys = bacteria enclosed in embryonic cells, mostly in lysis, fba = free bacteria in peripheral layer (Fig. 4: light microscopy; Fig. 5: transmission electron microscopy).

for some thyasirid bivalves (Southward 1986), for the vestimentiferans *Riftia pachyptila* (Bosch & Grassé 1984a, b) and *Tevnia* sp. (Bassaglia 1986) and is comparable to the processes found in the root nodules of Leguminosae (Sprent 1979).

Thus, it seems that by their symbiosis with sulfur-oxidizing bacteria the worms have acquired a tripartite nutritional basis via transepidermal uptake of dissolved organic matter, utilization of bacterial metabolic products, and digestion of bacterial cells. This flexible trophic pattern seems to allow for the complete loss of digestive and excretory organs. Moreover, the worms gained access to a new habitat, the sulfidic sediments. Examination of four additional species within the approximately 40 species of gutless marine tubificids showed that in each case associations with subcuticular bacteria are established, suggesting that this symbiosis is geographically as ubiquitous as it is evolutionarily favorable.

An intricate, mutualistic and well-established symbiosis as seen in *P. leukodermatus* might be predicted to have evolved a fairly secure method of transmitting the bacterial partners through the generations of their hosts rather than depending on more or less randomly repeated *de-novo*-infections of the worms. A regular transmission would require specific adaptative trends in the reproductive biology of these oligochaetes (Giere & Langheld 1987).

In fact, investigation of fully mature worms reveals formation of a ventral "genital pad" (Fig. 4), a blister-like structure packed with masses of fully active bacteria ensheathed only by a thin cuticular envelope. In a few cases deposition of single eggs was observed. They are covered by a sticky mucus, but not enclosed in a cocoon as would be typical for oligochaetes. These eggs were already infected by the characteristic bacteria. These first bacteria are mostly intracellular, within the cleavage cells, and

clearly in a lytic stage (Fig. 5). Only a few peripheral bacteria maintain their subcuticular, extracellular position and have a healthy appearance. Probably the latter stock is the source of the rich supply of extracellular, active prokaryotes in adult worms. In fortuitous sections of freshly laid eggs, still adhering to the body, the bacteria appear to be just about to enter the egg membrane through deep invaginations. Since we found free bacteria only during egg deposition and the earliest developmental stages, we conclude that prokaryotic symbionts are released by rupture or, more probably, by lysis of the genital pad adjacent to the oviducal pore. Subsequently, they are transmitted by invasion of the freshly deposited egg.

Although some microbiological and zoological details are not yet fully understood, we envisage the following scenario: While in early stages a defense mechanism initially causes intracellular lysis of the bacterial intruders, a balance between production of extracellular bacteria and their intracellular digestion becomes established in the growing worms, resulting in a coordinated symbiotic relationship between these aberrant marine tubificids and chemoautotrophic sulfur bacteria.

Acknowledgments

Literature Cited

Bassaglia, Y. 1986. Le trophosome d'un Vestimentifère. Structure et physiologie d'une symbiose. —Université Paul & Marie Curie, D.E.A. d'Océnologie Biologique Paris, 42 pp. + 18 pp. of appendices and bibliography + 8 pls.

Bosch, C., & P.-P. Grassé. 1984a. Cycle partiel des bactéries chimiautotrophes symbiotiques et leurs rapports avec les bactériocytes chez *Riftia pachyptila* Jones (Pogonophore Vestimentifère). I. Le trophosome et les bactériocytes.—Comptes Rendus de l'Académie des Sciences, Paris 299 (sér. 3):371–376.

———. 1984b. Cycle partiel des bactéries chimiautotrophes symbiotiques et leurs rapports avec les bactériocytes chez *Riftia pachyptila* (Pogonophore Vestimentifère). II. L'évolution des bactéries symbiotiques et des bactériocytes.—Comptes Rendus de l'Académie des Sciences, Paris 299 (sér. 3):413–419.

Cavanaugh, C. M., S. L. Gardiner, M. L. Jones, H. W. Jannasch, & J. B. Waterbury. 1981. Prokaryotic cells in the hydrothermal vent tube worm *Riftia pachyptila* Jones: Possible chemoautotrophic symbionts.—Science 213 (4505):340–342.

Felbeck, H., G. Liebezeit, R. Dawson, & O. Giere. 1983. CO$_2$ fixation in tissues of marine oligochaetes (*Phallodrilus leukodermatus* and *P. planus*) containing symbiotic, chemoautotrophic bacteria.—Marine Biology 75:187–191.

Giere, O. 1979. Studies on marine Oligochaeta from Bermuda, with emphasis on new *Phallodrilus*-species (Tubificidae).—Cahiers de Biologie Marine 20:301–314.

———; H. Felbeck, R. Dawson, & G. Liebezeit. 1984. The gutless oligochaete *Phallodrilus leukodermatus* Giere, a tubificid of structural, ecological and physiological relevance.—Hydrobiologia 115:83–89.

———, & C. Langheld. 1987. Structural organisation, transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes.—Marine Biology 93:641–650.

———, G. Liebezeit, & R. Dawson. 1982. Habitat conditions and distribution pattern of the gutless oligochaete *Phallodrilus leukodermatus*.—Marine Ecology Progress Series 8:291–299.

———, C. O. Wirsen, C. Schmidt, & H. W. Jannasch. 1988. Contrasting effects of sulfide and thiosulfate on symbiotic CO$_2$-assimilation of *Phallodrilus leukodermatus* (Annelida).—Marine Biology 97:413–419.

Liebezeit, G., H. Felbeck, R. Dawson, & O. Giere. 1983. Transepidermal uptake of dissolved car-

bohydrates by the gutless marine oligochaete *Phallodrilus leukodermatus* (Annelida).—Océanis 9:205–211.

Southward, E. C. 1986. Gill symbionts in thyasirid and other bivalve molluscs.—Journal of the Marine Biological Association of the United Kingdom 66:889–914.

Sprent, J. I. 1979. The biology of nitrogen-fixing organisms. McGraw-Hill Book Company, London, 196 pp.

Steudel, R. 1985. Neue Entwicklungen in der Chemie des Schwefels und des Selens.—Nova Acta Leopoldina, N.F. 59, No. 264:231–246.

———, G. Holdt, T. Gobel, & W. Hazeu. 1987. Chromatographische Trennung höherer Polythionate $S_nO_6^{2-}$ (n = 3 . . . 22) und deren Nachweis in Kulturen von *Thiobacillus ferrooxidans*; molekulare Zusammensetzung bakterieller Schwefelausscheidungen.—Angewandte Chemie 99:143–146.

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